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International Application No.:	PCT/US04/11919	Applicant:	NORWOOD IMMUNOLOGY, LTD.
Applicant's file reference	286336.160WO	International Filing Date:	April 19, 2004
Title:	**Tolerance to Graft Prior to Thymic Reactivation**		

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AMENDMENT UNDER ARTICLE 19

LETTER (Section 205(b))

Applicant respectfully requests that the claims as filed be replaced with the appended amended claims. The differences between the claims as filed and the claims as amended are indicated below.

As originally filed the application had 27 claims; as currently amended it has 31 claims. Claims 1-3, 7, 15, 20-23, 25, and 27 have been replaced by amended claims bearing the same numbers. Claim 26 has been canceled without prejudice. New claims 28-32 have been added.

STATEMENT UNDER ARTICLE 19(1) (Rule 46.4)

Applicant respectfully requests that the claims as filed be replaced with the appended amended claims on the replacement sheets. Applicant respectfully submits that former claims 1-27 have been amended without prejudice to applicant's right to prosecute any subject matter no longer being claimed. Claims 1-3, 7, 15, 20-23, 25, and 27 have been replaced by amended claims bearing the same numbers. Claim 26 has been canceled. Claims 28-32 have been added.

Independent claims 1-3 have been amended, *inter alia*, to add the step "analyzing the reactivity of T cells of the patient to cells from the donor or MHC matched to the donor, wherein the T cells have decreased responsiveness to donor or MHC matched cells compared to the responsiveness of the patient's T cells prior to treatment." Claim 7 and 22 have been amended to correct incorrect claim dependency. Claims 13-15 and 20-23 have been amended to add additional members to the Markush groups recited in these claims.

Applicant respectfully submits that amended claims 1-32 submitted herewith are fully supported by the description in accord with the requirements of Articles 5 and 6 of the Patent Cooperation Treaty. Applicant submits that the presently claimed subject matter meets the requirements of novelty, inventive step, and industrial applicability.

Respectfully submitted,

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REPLACEMENT SHEETS

1. A method for inducing tolerance in a patient to a graft from a mismatched donor, comprising:

depleting immune cells of the patient;

disrupting sex steroid-mediated signaling in the patient;

administering cells from the donor to the patient, wherein the cells are selected from the group consisting of stem cells, progenitor cells, and combinations thereof; and

analyzing the reactivity of T cells of the patient to cells from the donor or MHC matched to the donor, wherein the T cells have decreased responsiveness to donor or MHC matched cells compared to the responsiveness of the patient's T cells prior to treatment,

wherein the patient has increased tolerance to ~~a~~ the donor graft compared to an untreated patient.

2. A method for inducing tolerance in a patient to a graft from a mismatched donor, comprising:

depleting immune cells of the patient;

disrupting sex steroid-mediated signaling in the patient, wherein the functionality of the bone marrow of the patient is increased without, prior to, or concurrently with, reactivation of the patient's thymus;

administering cells from the donor to the patient, wherein the cells are selected from the group consisting of stem cells, progenitor cells, and combinations thereof;and

analyzing the reactivity of T cells of the patient to cells from the donor or MHC matched to the donor, wherein the T cells have decreased responsiveness to donor or MHC

matched cells compared to the responsiveness of the patient's T cells prior to treatment,

wherein the patient has increased tolerance to the donor graft compared to an untreated patient.

3. A method for inducing tolerance in a patient to a graft from a mismatched donor, comprising:

depleting immune cells of the patient;

disrupting sex steroid-mediated signaling in the patient;

administering cells from the donor to the patient, wherein the cells are selected from the group consisting of stem cells, progenitor cells, and combinations thereof;

allowing donor cell engraftment in the patient's bone marrow, wherein the donor cell engraftment is enhanced without, prior to, or concurrently with thymus reactivation;and

analyzing the reactivity of T cells of the patient to cells from the donor or MHC matched to the donor, wherein the T cells have decreased responsiveness to donor or MHC matched cells compared to the responsiveness of the patient's T cells prior to treatment,

wherein the patient has increased tolerance to the donor graft compared to an untreated patient.

4. The method of any one of claims 1-3, wherein the thymus of the patient has been at least in part atrophied.

5. The method of claim 4, wherein the patient has a disease that at least in part atrophied the thymus of the patient.

6. The method of claim 4, wherein the patient has had a treatment of a disease that at least in part atrophied the thymus of the patient.

7. The method of claim 6, wherein the treatment of the disease is immunosuppression, chemotherapy, or radiation treatment.

8. The method of any one of claims 1-3, wherein the stem cells are selected from the group consisting of hematopoietic stem cells, epithelial stem cells, and combinations thereof.

9. The method of any one of claims 1-3, wherein the progenitor cells are selected from the group consisting of lymphoid progenitor cells, myeloid progenitor cells, and combinations thereof.

10. The method of claim 8, wherein the cells are hematopoietic stem cells.

11. The method of claim 10, wherein the hematopoietic stem cells are $CD34^+$.

12. The method of claim 1 or 2, wherein the cells are administered at the time disruption of sex steroid-mediated signaling is begun.

13. The method of any one of claims 1-3, further comprising administering to the patient a substance selected from the group consisting of at least one cytokine, at least one hematopoietin, at least one lymphokine, at least one interleukin, at least one CSF, at least one growth factor, and a combination thereof.

14. The method of claim 13, wherein the cytokine is selected from the group consisting of Interleukin 1 (IL-1), Interleukin 2 (IL-2), Interleukin 3 (IL-3), Interleukin 4 (IL-4), Interleukin 5

(IL-5), Interleukin 6 (IL-6), Interleukin 7 (IL-7), Interleukin 8 (IL-8), Interleukin 9 (IL-9), Interleukin 10 (IL-10), Interleukin 11 (IL-11), Interleukin 12 (IL-12), Interleukin 13 (IL-13), Interleukin 15 (IL-15), Interferon gamma (IFN-γ), and combinations thereof.

15. The method of claim 13, wherein the growth factor is selected from the group consisting of members of the epithelial growth factor family, members of the fibroblast growth factor family, stem cell factor, granulocyte colony stimulating factor (G-CSF), keratinocyte growth factor (KGF), granulocyte-macrophage colony stimulating factor (GM-CSF), insulin-like growth factor-1 (IGF-1), a growth hormone, a thyroid hormone, M-CSF, Meg-CSF, MIF, LIF, TNF, PDGF, human growth hormone, B cell growth factor, B cell differentiation factor, eosinophil differentiation factor, and combinations thereof.

16. The method of any one of claims 1-3, wherein the sex steroid-mediated signaling is disrupted by castration.

17. The method of claim 16, wherein the sex steroid-mediated signaling is disrupted by surgical castration.

18. The method of claim 16, wherein the sex steroid-mediated signaling is disrupted by chemical castration.

19. The method of claim 18, wherein the sex steroid-mediated signaling is disrupted by administration of one or more pharmaceuticals.

20. The method of claim 19, wherein the one or more pharmaceuticals is selected from the group consisting of LHRH agonists, LHRH antagonists, anti-LHRH vaccines, anti-androgens, anti-estrogens, SERMs, SARMs, SPRMs, ERDs, aromatase inhibitors, adrenal gland blockers, aldosterone antagonists, antiprogestogens, progestins, antiprogestins, dioxalan derivatives, and combinations thereof.

21. The method of claim 20, wherein the LHRH agonists are selected from the group consisting of goserelin, leuprolide, lupron, triptorelin, meterelin, buserelin, histrelin, nafarelin, lutrelin, leuprorelin, deslorelin, cystorelin, decapeptyl, gonadorelin, and acetates, citrates and other salts thereof, and combinations thereof.

22. The method of claim 20, wherein the LHRH antagonists are selected from the group consisting of abarelix, cetrorelix, acetates, citrates, and other salts thereof, and combinations thereof.

23. The method of claim 20, wherein the anti-androgen is selected from the group consisting of Cosudex®, bicalutamide, cyproterone acetate, liarozole, ketoconazole, flutamide, megestrol acetate, dutasteride, finasteride, eulexin, and combinations thereof.

24. The method of claim 20, wherein the anti-estrogen is selected from the group consisting of anastrozole, fulvestrant, tamoxifen, clomiphene, diethylstilbestrol, diethylstilbestrol diphosphate, danazol, droloxifene, iodoxyfene, toremifene, raloxofene, and combinations thereof.

25. The method of claim 20, wherein the adrenal gland blocker is selected from the group consisting of aminoglutethimide, formestane, vorazole, exemestane, anastrozole, letrozole, exemestane, and combinations thereof.

26. (Canceled)

27. The method of any one of claims 1-3, wherein the donor graft is selected from the group consisting of cell of the donor, tissues of the donor, organs of the donor, and combinations thereof.

28. The method of any one of claims 1-3, wherein the sex steroid-mediated signaling to the thymus is disrupted by lowering the level of a sex steroid hormone.

29. The method of any one of claims 1-3, where the cells from the mismatched donor are genetically modified.

30. The method of any one of claims 1-3, wherein the method results in the generation of a chimera selected from the group consisting of a chimeric thymus, chimeric hemopoietic cells, chimeric lymphoid cells, chimeric T cells, chimeric B cells, chimeric dendritic cells, a chimeric lymphoid organ, and any combination thereof.

31. The method of any one of claims 1-3, further comprising an allograft transplantation of a graft having the same histocompatibility as that of the mismatched donor to the patient.

32. The method of any one of claims 1-3, wherein the method comprises collecting blood samples from the patient from about 2 days to about 21 days after administering cells from the donor to the patient.